OBSERVATIONS ON A POPLAR LEAF INFECTION OCCURRING IN NORTH-EAST CHINA: THE GREY SPOT DISEASE *

Michel MORELET ** and Pierre SIGAUD ***

** Laboratory of Forest Tree Pathology, INRA, 54280 Champenoux, France
*** North-eastern Research Station, ONF, Parc de Haye, 54840 Velaine en Haye, France

ABSTRACT — A disease affecting poplar leaves and shoots has been reported in north-eastern China. Local field observations and identification carried out from 1991 to 1993 match Chinese literature on the alleged infecting agent, *Coryneum populinum*, an asexual form of the fungus *Mycosphaerella mandshurica*. However, taxonomic surveys undertaken in INRA-Nancy (France) do not confirm the identity of the former binomial nor the connection with the latter: samples examined and cultivated in France correspond to *Pollaccia mandshurica*, the asexual form of a new species, which has previously been described as *Venturia mandshurica*.

RÉSUMÉ — L'étude in situ, puis au laboratoire, de la maladie des taches grises qui affecte les feuilles et les pousses des Peupliers dans le nord-est de la Chine, a été entreprise par les auteurs de 1991 à 1993. Cette maladie est attribuée par les auteurs chinois au champignon *Coryneum populinum*, anamorphe de *Mycosphaerella mandshurica*. Les observations de terrain corroborent les études symptomatologiques et épidémiologiques publiées par ces auteurs. En revanche, l'étude microscopique et culturale de l'agent isolé des lésions, ainsi que l'établissement de son cycle vital, montrent que nous sommes en présence d'une tavelure typique, due à *Pollaccia mandshurica* (= *Coryneum populinum sensu* Ju *et al.* non sensu Bresadola) dont la téléomorphe est *Venturia mandshurica*.

KEY WORDS: *Coryneum populinum, Mycosphaerella mandshurica, Pollaccia mandshurica , Venturia mandshurica, Populus simonii, Populus simonii* x *P. nigra*, grey spot disease, scab, poplar, leaves, shoots, China.

INTRODUCTION

NATURAL CONDITIONS

North-eastern China is a vast territory formerly referred to as Manchuria, now divided into the three Provinces of Liaoning, Jilin and Heilongjiang.

* Most results of this paper were presented at the 37 th session of the International Poplar Commission (Izmit, Turkey, October 1994) which proceedings were not published.

Its centre is occupied by the Northeastern Plain, bordered by mountain ranges in the west, the north and the east, and open to the Yellow Sea in the south.

Field observations stated in this paper took place in the Korqing area, a piece of land stretching across the common border of Liaoning, Jilin and Inner Mongolia, between latitudes 41-44° N. and longitudes 117-125° E.

The climate is typically continental, with a semihumid influence in the south, and a pronounced trend towards aridity in the north-west. Mean annual rainfall varies from 350 mm to 550 mm, with important variations from year to year. More than two-thirds of the annual rainfall occurs from mid June to mid August.

Evaporation is intense (1300 mm to 2100 mm annually, i.e. from twice to six times the annual precipitation), linked to permanent winds (mean annual speed from 3.5 m/s to 4.5 m/s), which become dry sand storms during spring (April to June).

Average annual temperatures range from 5°C to 9°C according to the location, for a growing season of 150 to 170 frost-free days. January is the coldest month (mean annual temperature of −9°C to −16°C, with absolute minima down to −33°C), while July is the hottest (monthly average of 24°C, with an absolute maximum of 40°C).

Four seasons are therefore clearly marked, with dry and cold winters, dry and windy springs, temperate and rainy summers, and temperate but dry autumns.

Altitude is low to moderate, ranging from 15 m in the south to 270 m in the northwest.

Soils are generally formed by quaternary sandy deposits, with local patches of dark clays in lowlands or along rivers. pH values are always high, above 7, reaching 10 in alkaline lowlands.

Natural vegetation is typical of grasslands, with scarce forest relics of poplar and willow along flat banks of large rivers, or even temperate deciduous mixed relic stands of oak, elm and alder along deep narrow gorges. Artificial stands have been widely planted since the 1950's for land reclamation and soil protection, and most of the area has been afforested under the so-called "Three North Sherterbelt Afforestation Programme". Poplar is the main species used in those stands,in which both native balsams (*Populus simonii, Populus pseudosimonii*) and more recent hybrid selections (*Populus simonii* x *Populus nigra*) can be found.

Former studies and achievements

Grey-spot disease has been reported endemic in Hebei, Henan, Shaanxi, Xinjiang, Heilongjiang, Jilin and Liaoning, the most serious attacks occurring in the Northeast (Anonymous, 1984, 1987, 1988).

Many poplar clones or species are scored as susceptible, such as: *Populus simonii, P. pseudosimonii, P. cathayana, P. davidiana, P. nigra var. thevestina, P. nigra var. italica, P.* x *berolinensis, P.* x *simonigra* var. *baicheng 41, P.* x *simonigra* var. *Xiaohei* (Anonymous, 1984, 1987, 1988), along with other broadleaved species such as *Betula sp* (?) (Anonymous, 1988).

Some other clones or species are ranked as relatively resistant to the grey-spot disease: they include *Populus alba, P. bolleana, P. x euramericana var. Robusta, P.* x *euramericana var. canadensis, P.* x *euramericana* (?) var. *Leader Stalin, P. deltoides* (?) var. *Shanhaiguan, P. davidiana* x *bolleana*, (Anonymous, 1984, 1987, 1988).

The disease was mentioned as early as 1960 and the associated pathogen was described, illustrated and identified as *Coryneum populinum* Bresadola in Ju *et al.*

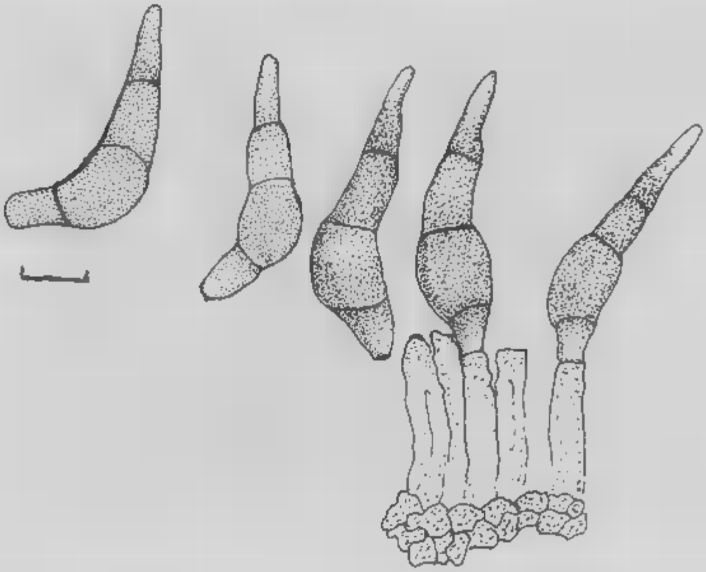

Fig. 1 — "*Coryneum populinum*" *sensu* JU *et al.*: conidia and conidiophores from living leaves of *Populus simonii* (?) (redrawn from ZHAO Li Ping, in Anonymous, 1984) (bar, 10 µm). Conidies et conidiophores de *Coryneum populinum* au sens de JU *et al.* sur feuilles vivantes de *Populus simonii* (?) (d'aprés ZHAO Li Ping, in Anonymous, 1984) (échelle, 10 µm)

(1965) (Fig. 1). Since then, it has been studied mainly in Liaoning and Heilongjiang but with *Mycosphaerella mandshurica* Miura as the alleged infecting agent. It was considered to be the teleomorph of a *Coryneum populinum sensu* Ju *et al.* (*sub nom. Coryneum populinum* Bres.) (Anonymous, 1984).

The main Chinese studies have focused on accurate description and identification of the parasite (Anonymous, 1984, 1987, 1988; Ju *et al.*, 1965; Xiang *et al.*,1988), on laboratory cultivation (Xiang *et al.*,1986), on comparing susceptibility among clones (Xiang *et al.*, 1986), on scoring of injuries (Hao *et al.*, 1991), on the dynamics and the prediction of epidemic cycle (Xu *et al.*, 1991 a & b), and on early detection of clonal resistance, etc.

The following paragraph will review field observations undertaken from 1991 to 1993. All are fully in accordance with relevant Chinese literature.

Field observations of infections by the grey spot disease

For most poplar clones, flushing occurs from mid April to early May, in both nursery and plantation. The very first pathological symptoms on the leaf do not appear before late May. In many places, these patterns have been noticed from one week to ten days after a sudden fall in day-time temperature. This is generally linked to localized rainfall, at a time when air temperatures rise rapidly during the day.

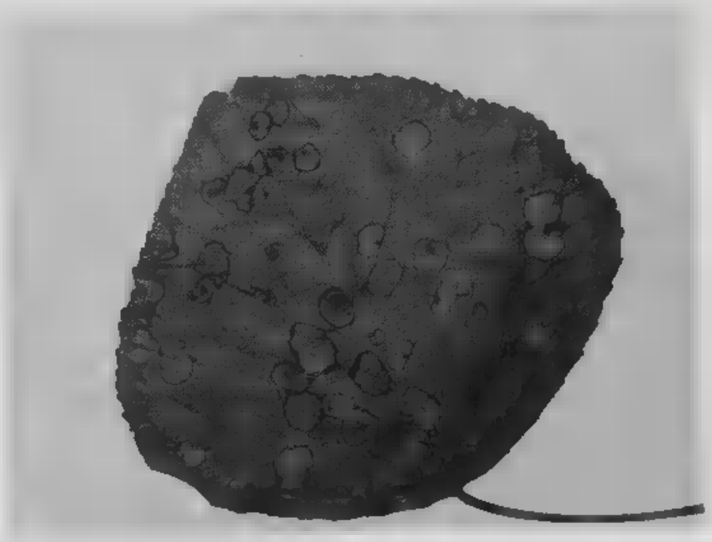

Fig. 2 — The grey spot disease: leaf injuries on *Populus simonii* x *P. nigra*. Maladie des taches grises: symptômes sur feuille de *Populus simonii* x *P. nigra*.

Fig. 3 — The grey spot disease: shoot injuries with a typical "charred" hook. Maladie des taches grises: attaque sur jeune pousse qui se dessèche et se recourbe en forme de crochet.

In nurseries, initial infections are noticed on basal leaves of two — or three-year old plants. At that point, poplar leaves are usually small-sized, thick and dark. Brown, circular, 5 mm to 1 cm-wide spots appear on the upper surface of the leaf. After one to two weeks, they form a depressed area, with a white-grey (silvery) characteristic colour inside, bordered by a dark brown margin (Fig. 2). At this stage, damage is limited: the surface of the blotches does not seem to increase significantly, and injuries seldom lead to leaf abcission.

After a few days, some black cracks burst into dark points on the smooth silvery surface, indicating the development of a new generation of conidia (Fig. 2).

By the middle of June, at the beginning of the rainy season, new infectious patterns appear on the surface of young soft tissues, developing leaves or tender terminal tips.

Irregular blotches with dark margins covered by a dark olive-green powder develop rapidly, warping the growing material, leading within two to three days to a complete decay of the organ. When a petiole is damaged, the leaf dies and falls off.

Damage affecting terminal tips or buds is much more serious, since it generally necroses the whole growing zone of soft green tissues. The apex of the shoot turns to a withered black hook characteristic of the disease. The apical part looks as if it had been charred (Fig. 3).

During the rainy season (mid June to mid August), the disease can therefore display different patterns according to the infected area: from the stem to the tip, along a young twig, circular, black-margined silvery spots, generally on small leaves, then scattered or coalescent dark olive-green irregular blotches on larger summer leaves, and even a black "charred" hooked apex can be noticed.

These symptoms may vary slightly from clone to clone.

According to the literature, the fungus winters in conidia form in the litter (leaves and twigs fallen on the ground) or on the bark of the branches. Wind, rain splashes propagate this material and when minimum requirements in terms of temperature and humidity are met, conidia are able to germinate and thus infect a new area (Xiang *et al.*, 1986, 1988).

In Heilongjiang, a clinal relationship has been shown between air humidity in summer and intensity of damage to leaves and young shoots, matching the corresponding periods of conidia production (Xu *et al.*, 1991a).

Studies seem to indicate peak infection in early July, while by late August few new blotches are to be expected. However, sudden outbreaks can be noticed in the field at this time, and they can be traced easily since they are separated by an undamaged area along the branch (Xu *et al.*, 1991 a).

Damage is obviously much more serious in the nursery than in mature plantations. In some nurseries, where inadequate space does not allow for crop rotation, and where poplars have been cultivated on the same site for many years, there have been reports of nearly complete destruction by the parasite.

Young plants up to age 5 or 6 can be heavily affected in the plantation.

The disease does not generally kill infected trees. The damaged leaves are still functional but photosynthesis is reduced, and vigour therefore decreased. Some studies estimate that up to 73 % of the annual growth may be lost at age 1 (Hao *et al.*, 1991). Weaker trees at the end of the first growing season are more likely to suffer from frost injuries during winter, and from other pest and insect hazards the following year.

Susceptibility / resistance to the disease seems under strong genetic control with high inheritability; the following surveys were made on different clones in 1991:

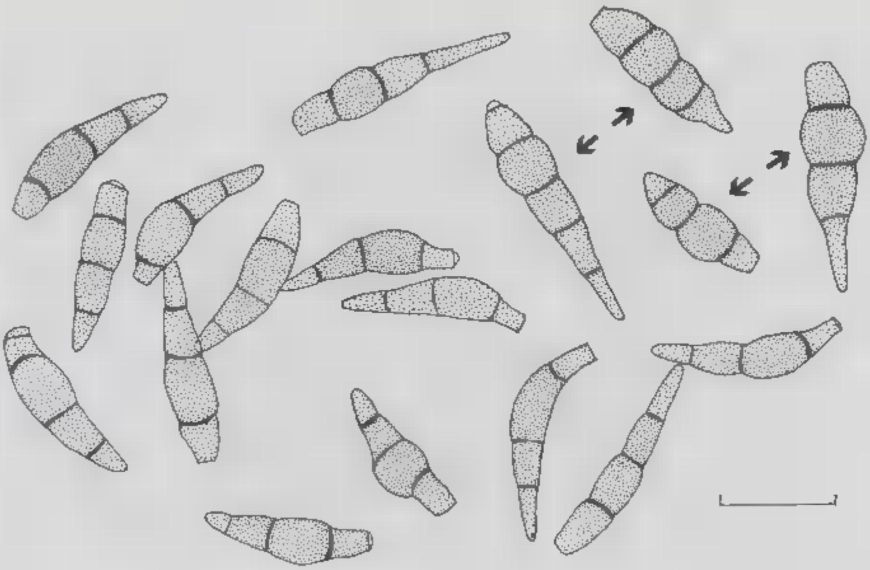

Fig. 4 — *Pollaccia mandshurica* (PFN 1466, holotype): mixture of conidia from shoots (arrows) and living leaves of *Populus simonii* x *P. nigra* (bar, 20 µm). Conidies de l'holotype de *P. mandshurica* prélevées sur pousse (flèches) et feuilles vivantes de *Populus simonii* x *P. nigra* (échelle, 20 µm).

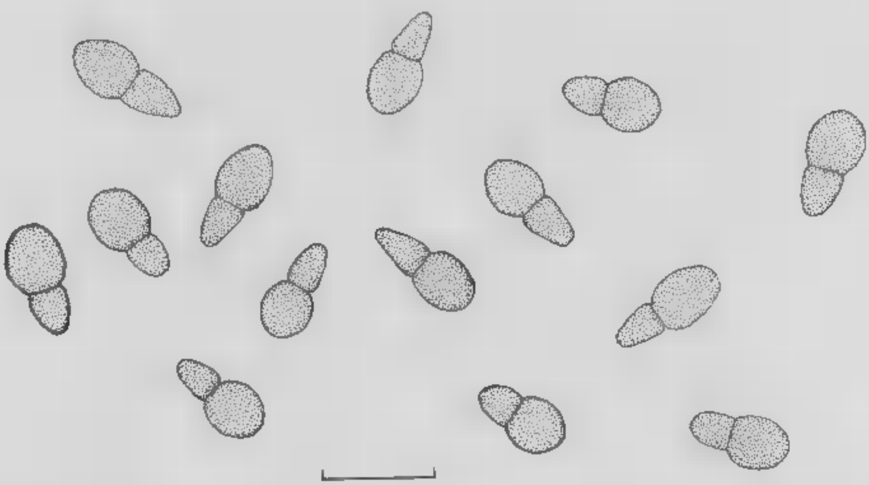

Fig. 5 — *Venturia mandshurica* (PFN 1470, holotype): mature ascospores ejected onto a slide from ascomata on dead leaves of *Populus simonii* x *P. nigra* (bar, 20 µm). Ascospores mûres de l'holotype de *V. mandshurica* projetées sur lame à partir d'ascocarpes sur feuilles mortes de *Populus simonii* x *P. nigra* (échelle, 20 µm).

All *P.* x *euramericana* hybrids, *P. deltoides*, *P. maximowiczii* or *P. trichocarpa* progenies display a very high, if not complete, resistance to the infection, at nursery stage (age 1 or 2). Most *P. simonii, P. nigra s.l.* and their hybrids *P.* x *simonigra* show a moderate to high susceptibility.

It is not known whether resistance to the parasite was taken into account in the Chinese poplar breeding programme in the 1960's.

Many of the pathological patterns and symptoms reported above are very similar to the aspen scab caused by *Venturia tremulae* Aderhold (anamorph: *Pollaccia radiosa* (Lib.) Bald. et Cif.) in Europe and North America (Morelet 1983).

Taxonomic examination

Infected leaves and shoots displaying morphological patterns of the grey-spot disease and originating from Liaoning were examined in summer, 1992 in the INRA laboratories in Nancy, France.

In addition, some damaged leaves wintered in 1992/1993 in Inner Mongolia on the ground surface of a production nursery were studied in 1993.

The results of the microscopic examination were as follows:

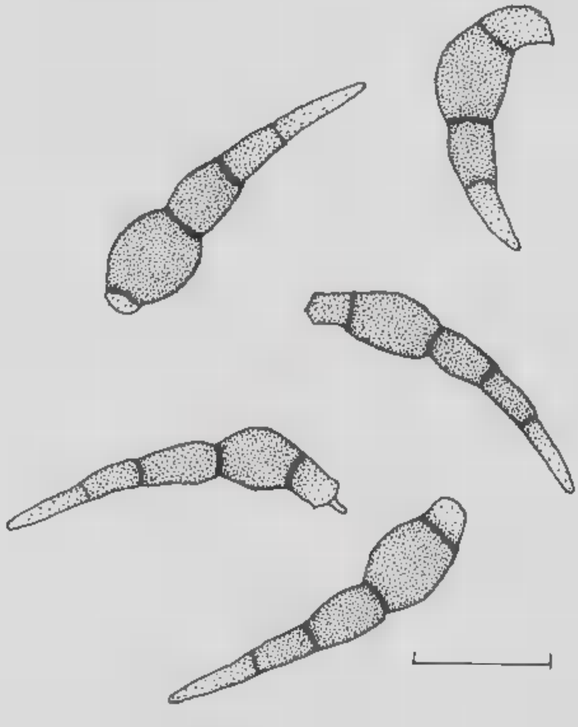

Fig. 6 - *Pollaccia mandshurica*: conidia from monoascospore culture of *Venturia mandshurica* (PFN 1470, holotype) (bar, 20 μm). Conidies de *Pollacia mandshurica* obtenues *in vitro* à partir d'une souche monoascospore de *Venturia mandshurica* (échelle, 20 μm).

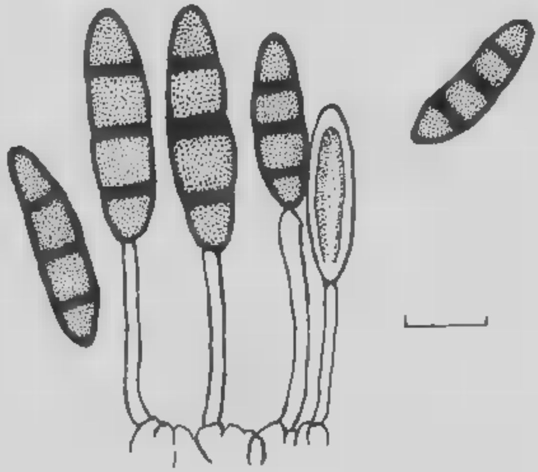

Fig. 7 — *Coryneum populinum* Bresadola: conidia and conidiophores from bark of *Populus nigra* (redrawn from Bresadola 1892) (bar, about 10 µm). Conidies et conidiophores de *Coryneum populinum* sur écorce de *Populus nigra* (d'aprés Bresadola 1892) (échelle, environ 10 µm).

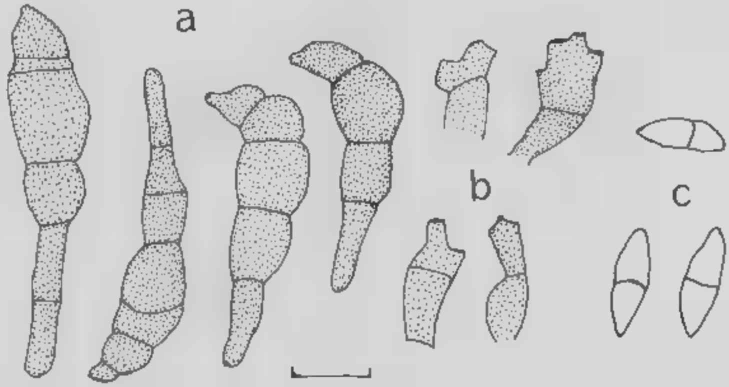

Fig. 8 — *Mycosphaerella mandshurica* Miura: conidia (a), conidiophores (b) and ascospores (c) from leaves of *Populus laurifolia* (redrawn from Miura 1928) (bar, 10 µm). Conidies (a), conidiophores (b) et ascospores (c) de *Mycosphaerella mandshurica* sur feuilles de *Populus laurifolia* (d'aprés Miura 1928) (échelle, 10 µm).

— from the spots on living leaves: black, abundant, intraepidermic then subcuticular epiphyllous stromatic acervuli. Conidiophores formed from the upper cells of the stroma, erect, cylindrical, pale brown, 1-2 septate, up to 17 µm. long. Conidiogenous cells cylindrical, with up to 3 annellations (in pure culture) but usually with a single jagged collarette. Conidia fusiform, often curved, tapered towards an obtuse to subacute apex, less tapered towards a truncate base, pale brown, (1)-2-3-(4-5) euseptate (mainly 3-septate), constricted at the septa, 24-39 × 6,6-9,9 µm.(Fig. 4). In accordance with these observations, typical of the genus *Pollaccia* Bald. et Cif., and after a comparative study of all species recognized in this genus, (from *Populus*), Morelet (1993) proposed for this taxon the binomial: *Pollaccia mandshurica* as a new species. Isotype in PC Herbarium.

— from wintered leaves: pseudothecia immersed, subepidermal, globose, with a papillate ostiole set with septate, brown setae, up to 133 µm long. Asci cylindrical to oblong, short stalked, bitunicate, 8-spored, 84-120 × 16,6-18,5 µm. Ascospores pale yellow, broadly elliptical, septate in the lower third, constricted at the septum, smooth, 18-22 × 9-11 µm (Fig.5). This teleomorph, typical of the genus *Venturia* Sacc., was described as a new species: *Venturia mandshurica* Morelet (Isotype in PC Herbarium), and the connection with *Pollaccia mandshurica* was ascertained in pure culture by the development of the latter morph (Fig.6) from single ascospore inoculum (Morelet, 1993). Strains are preserved in LCP 95.3820, MUCL 39221, MPFN 307.

Concerning *Coryneum populinum* and *Mycosphaerella mandshurica*:

A comparison of descriptions and illustrations of *Coryneum populinum sensu* Ju *et al*. (Fig. 1) shows that they are different from *Coryneum populinum* Bres. (Fig.7) described on the bark of a *Populus nigra* trunk near Trent (Italy) by Bresadola (1892). According to Sutton (1975) the latter species looks very much like a *Seimatosporium* Cda. *Coryneum populinum sensu* Ju *et al*. is neither a *Coryneum* Nees ex Schw., nor a *Seimatosporium* but undoubtedly a *Pollaccia*, similar to *P. mandshurica*. Therefore it is also different from the first unnamed anamorph of *Mycosphaerella mandshurica* (cf. infra) and the connection between them reported by Xiang *et al*. (1988) is questionable.

An attempt to obtain Miura's specimens of *Mycosphaerella mandshurica* was not successful. But judging by the description Miura gave of this fungus (Miura, 1928), it seems that he described two unnamed anamorphs associated to the teleomorph, but without giving experimental evidence for affiliation.

The first one shows some similarities in conidial shape with *Pollaccia mandshurica* (Fig. 8a), but noticeable differences in sympodial conidiogenous cells (Fig. 8b). The second anamorph is probably an *Asteromella* state of the *Mycosphaerella*.

Finally, *Mycosphaerella mandshurica* is the most appropriate name for the teleomorph, and therefore should not be confused with *Venturia mandshurica*.

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